ETEC colonisation factors disrupt the antigen presenting capacity of porcine intestinal dendritic cells

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INTRODUCTION

Enterotoxigenic *E. coli* (ETEC) are not only a major cause of diarrhoea in travellers to and children in developing countries, but also cause neonatal and postweaning diarrhoea in piglets, leading to a reduced feed conversion and a higher mortality rate. As a consequence ETEC infections result in severe economic losses in the swine production industry. This intestinal pathogen displays colonisation factors or fimbriae on its surface enabling the microorganism to adhere to the intestinal epithelium (Fig. 1). In pig, F4 and F18 fimbriae are the most frequently associated with ETEC-induced diarrhoea[1]. As opposed to F4 fimbriae, oral immunisation with F18 fimbriae doesn't protect piglets from a subsequent challenge infection[2]. F18 fimbriae bind glycosphingolipids in the apical membrane of enterocytes, but no transcytosis occurs, resulting in lower sunepithelial antigen concentrations as compared to F4 fimbriae, which bind the transcytotic receptor aminopeptidase N[3,4]. However, M-cell mediated transport of F18 fimbriae should still occur. Hence, besides a lower antigen concentration, these fimbriae could affect the function of intestinal antigen presenting cells. Here, we investigated the influence of purified F18 fimbriae on the antigen presentation capacity of small intestinal lamina propria dendritic cells (LPDCs).

RESULTS AND DISCUSSION

Fimbriae were purified from F4$^+$ and F18$^+$ ETEC strains and intestinal MCs from small intestinal lamina propria (jejunum) were isolated as previously described[5]. Based on the expression of MHCII, CD16 and CD172a several intestinal MC populations could be distinguished (Fig. 2A). We obtained two different LPAPCs populations: MHCII$^+$CD172a$^+$CD16^{hi} (CD16^{hi}) and MHCII$^+$CD172a$^+$CD16$^+$ (CD16$^+$). Although we could detect three CD172a$^+$ myeloid cell populations differing in CD16 expression, only the CD16^{hi} and CD16$^+$ populations are MHCII$^+$, while the CD172a$^+$CD16$^-$ population is MHCII$^-$ and represents intestinal eosinophils. Besides these CD172a$^+$ myeloid cells, a small CD172a$^-$CD16$^+$ cell population was present, which are CD3$^+$ T-cells (Fig. 2B). In addition, we could discern a CD172a$^-$MHCII$^+$ cell population, which mainly consist of IgM$^+$ B-cells (Fig. 2C). Morphological analysis revealed that as opposed to the CD16$^+$ population, CD16^{hi} cells have many vacuoles, a typical feature of macrophages (Fig. 2D). Besides their morphological appearance, these two populations also differ in their expression of aminopeptidase N (CD13, F4R) and CD11R1 (Fig. 2E).

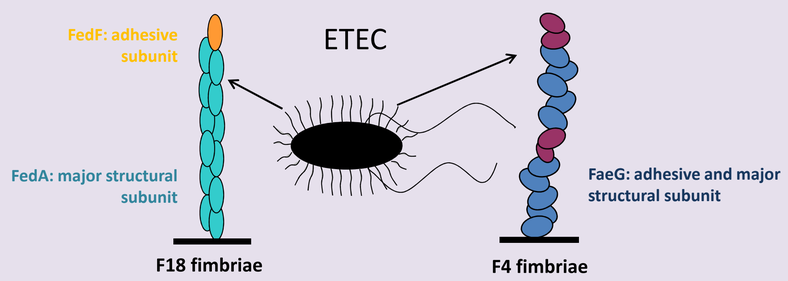

Fig. 1: Schematic representation of fimbriae present on porcine-specific ETEC strains. Colonization factors or fimbriae are long, polymeric finger-like appendages on the bacterial surface. F18 fimbriae are composed of the structural subunit FedA and the tip adhesin FedF. In contrast, in F4 fimbriae the major structural subunit FaeG also serves as the adhesin.

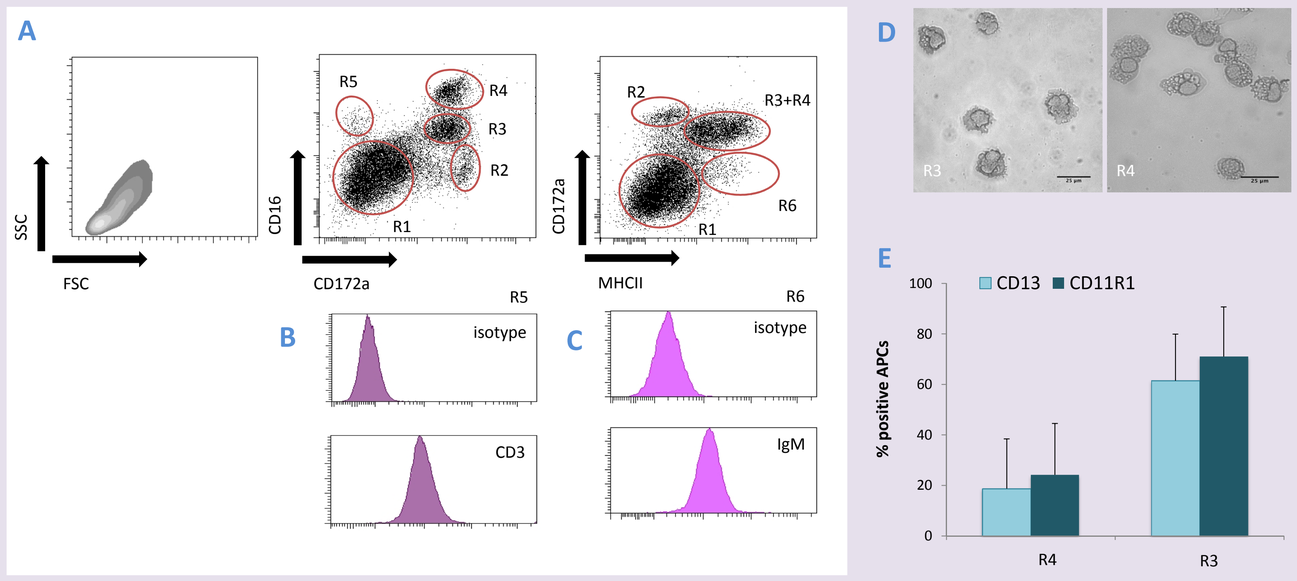

Fig. 2: Phenotypical analysis of porcine small intestinal lamina propria MCs. LPMCs were stained to determine CD172a, CD16 and MHCII surface expression by live LPMCs (Sytox Blue$^-$, doublet discrimination). **A)** All populations , except R1, were sorted with a FACSariaIII (BD biosciences). R1: lymphocytes; R2-R4: myeloid cells; R5: CD16$^+$ lymphocytes; R6: MHCII$^+$ lymphocytes. Representative dot plots for 4 separate experiments. **B-C)** R5 consists of CD3$^+$ T-cells, while the MHCII$^+$ lymphocytes in R6 are mainly IgM$^+$ B-cells. **D)** Morphological difference between the sorted R3 (CD16$^+$) and R4 (CD16^{hi}) LPAPC populations (n = 4). Bar = 25 µm. **E)** Phenotypical analysis of the CD16^{hi} and CD16$^+$ LPAPCs (n = 3).

While the CD16^{hi} cells have low CD13 and CD11R1 expression levels, the CD16$^+$ APCs express both markers at higher levels. In pig, the majority of the intestinal lymph cells are CD11R1$^+$.[6] Based on these data, we conclude that the CD16^{hi} cells resemble macrophages, while the CD16$^+$ population consists of dendritic cells.

To assess the effect of F18 fimbriae on the function of LPAPCs, F18 and F4 fimbriae-treated LPAPCs were cocultured with T-cells. In contrast to F4 fimbriae, F18 fimbriae significantly diminished the T-cell proliferation inducing capacity of CD16^{hi} and CD16$^+$ LPAPCs as compared to mock-treated LPAPCs (Fig. 3A). This inhibitory effect was not due to LPS present in the fimbrial preparations. Indeed, both in the presence of polymyxin B and upon removal of endotoxins (>90%) F18 fimbriae still drastically reduced the T-cell proliferation inducing capacity of CD16^{hi} and CD16$^+$ LPAPCs as compared to mock- or F4 fimbriae-stimulated LPAPCs (Fig. 3B).

Although the fimbriae were washed out from the LPAPCs cultures prior to coculture, we assayed the effect of F18 fimbriae on ConA-induced T-cell proliferation to exclude the possibility of a direct inhibitory effect of residual F18 fimbriae on T-cell proliferation. As shown in figure 3C, F18 fimbriae were unable to reduce T-cell proliferation as compared to controls.

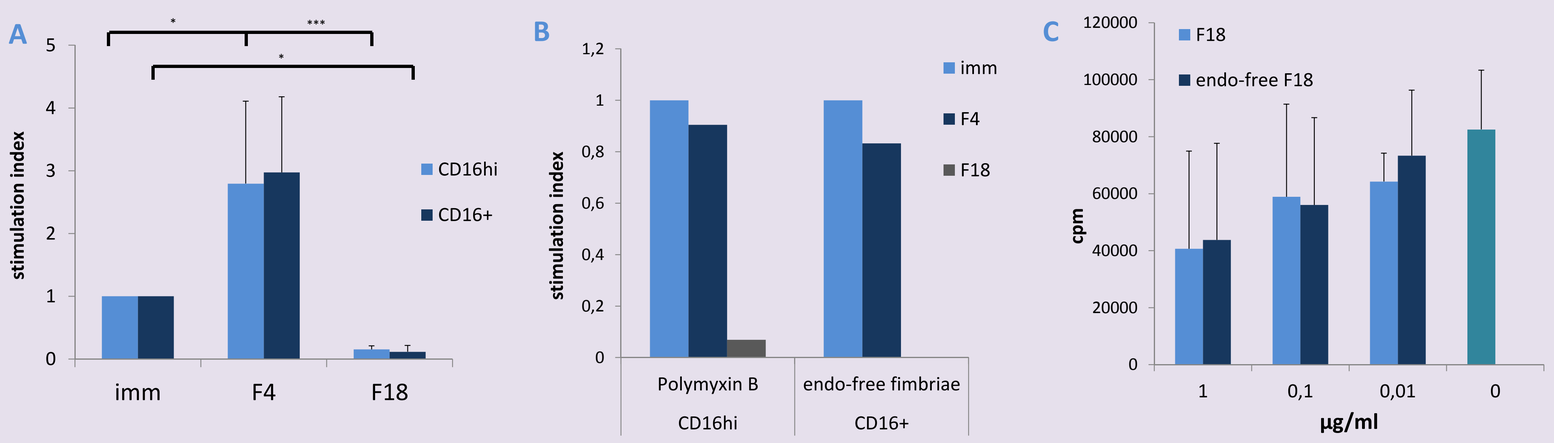

Fig. 3: F18 fimbriae disrupt the antigen presentation capacity of intestinal dendritic cells. **A)** Both CD16^{hi} and CD16$^+$ LPAPCs (1.0 x 10^4) were stimulated for 24 h with 1.0 µg/ml F4 or F18 fimbriae or mock-treated (imm) and then cocultured with 1.0 x 10^5 CD6$^+$ T-cells for 5 days in round-bottomed 96-well plates. Proliferative responses were measured via the incorporation of tritiated thymidin (CD16^{hi}, n = 3; CD16$^+$, n = 2). **B)** CD16^{hi} LPAPCs were stimulated with 1.0 µg/ml F4 or F18 fimbriae in the presence of polymyxin B (100 µg/ml). Alternatively, CD16$^+$ LPAPCs were stimulated with endotoxin-free fimbriae (1.0 µg/ml). Endotoxin present in the fimbrial preparations was removed with EndoTrap columns. Upon incubation for 24 h, LPAPCs were cocultured and the proliferative responses were measured as described above. The stimulation index was calculated by dividing the mean cpm (triplicate wells) of the stimulated conditions by the mean cpm of the mock-stimulated LPAPC-CD6$^+$ T-cell cocultures. **C)** CD6$^+$ T-cells were seeded in round-bottomed 96-well plates and incubated for 72 h with ConA (5 µg/ml) and different concentrations of F18 fimbriae or endotoxin-reduced F18 fimbriae (n = 2). cpm: counts per minute.

CONCLUSION

F18 fimbriae drastically diminished the antigen presentation capacity of porcine LPDCs. Although the exact mechanism is still unclear, we hypothesize that these F18 fimbriae disrupt lipid raft formation, resulting in a deformation of the immunological synapse necessary to induce T-cell activation. Indeed, F18 fimbriae adhere to glycosphingolipids and these are important for the formation of lipid rafts. Alternatively, F18 fimbriae could drive the maturation of LPDCs towards a tolerogenic phenotype, which in turn could lead to the differentiation of regulatory T-cells, capable of suppressing CD4$^+$ effector T-cell proliferation. Current efforts are on going the unravel the molecular mechanism of this fimbriae-mediated reduction of LPDCs-induced T-cell proliferation. These results could lead to the development of an improved vaccine against F18$^+$ ETEC and deepen our understanding of potential novel immune evasive mechanisms employed by bacterial pathogens.

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